



Express Mail No. EV 346 796 080 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Dov ZIPORI et al.

Confirmation No.: 5156

Application No.: 10/643,982

Group Art Unit: 1644

Filing Date: August 20, 2003

Examiner: M. Belyavskyi

For: IMMUNOGLOBULIN SUPERFAMILY
VARIANTS EXPRESSED IN
MESENCHYMAL CELLS AND
THERAPEUTIC USES THEREOF

Attorney Docket No.: 85189-5000

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Dov Zipori, hereby declare as follows:

1. I, Dov Zipori, a citizen of Israel residing at 35/4 Hirshenson Street, Rehovot 76484, Israel;
am one of the named inventors in the subject application and wish to submit information in support of the Amendment being submitted herewith.
2. I am familiar with the contents of the office action. I am a biologist by training and experience and have a Ph.D. in the field Cell Biology, from the Weizmann Institute of Science, Rehovot, Israel from 1976. I am currently employed by the assignee of this application and my current title is Associate Professor, Director of the Helen and Martin Kimmel Stem Cell Research Institute at the Weizmann Institute.
3. I discovered and characterized, for the first time, the expression of stro- μ chain in mesenchyme-derived cell lines. Figure 1 shows RT-PCR of a mesenchyme derived endothelial-like cell line (MBA-2.1) and primary embryo mesenchymal cells (mouse embryonic fibroblasts; MEF). The MBA-2.1 cell line and the primary MEF from wild type (W.T.) animals express μ chain, while IgM^{-/-} mice do not express a comparable mRNA transcript. Furthermore, no light chain mRNA

transcripts were detected, indicating that the μ chain mRNA does not originate from lymphocyte contamination of the stromal cultures.

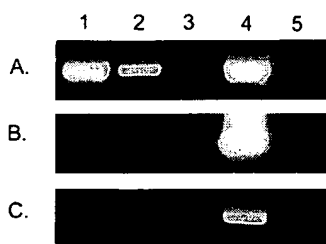


Figure 1 Expression of μ heavy chain as detected by RT-PCR in mouse stromal cells. RT-PCR analysis identifies μ heavy chain constant region (A), but no κ (B) or λ (C) light chains mRNA transcripts in (1) MBA-2.1 cells and (2) W.T. MEF (3). No such transcript is found in IgM^{-/-} MEF. (4) control spleen (5) DDW.

4. Northern blot analysis (Figure 2) verified the finding in Figure 1 and shows that the transcript in stromal cells is smaller than that found in spleen lymphocytes.

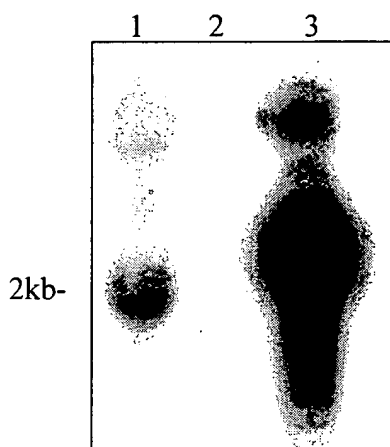


Figure 2 Northern blot analysis of truncated μ heavy chain in stromal cells. Northern blot analysis for μ heavy chain gene in stromal cells. Total RNA was extracted from (1) MBA-2.1 cells (2) IgM^{-/-} MEF (3) Spleen. The membrane was hybridized with a radiolabeled μ heavy chain constant region probe, washed and exposed to X-ray film.

5. Translation of the mRNA transcript into a mature protein was demonstrated by Western blot using specific antibodies to the constant region of the μ heavy chain (Figure 3). We thus conclude that mesenchymal cells express a truncated form of the μ heavy chain, designated herein stro- μ .

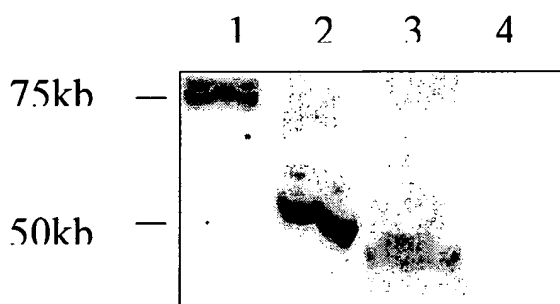
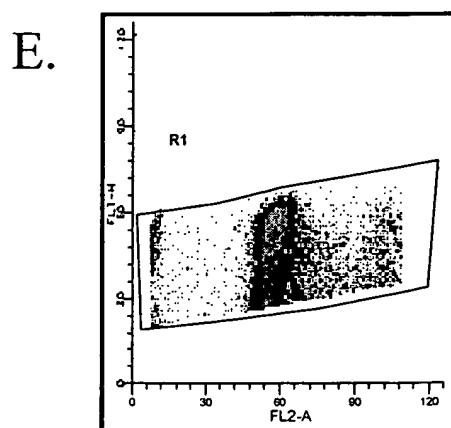
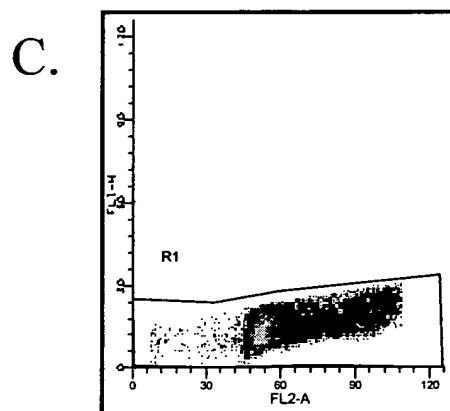
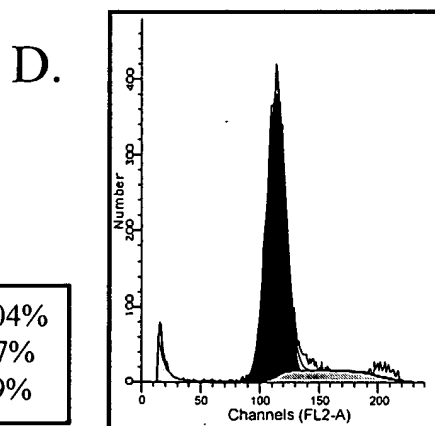
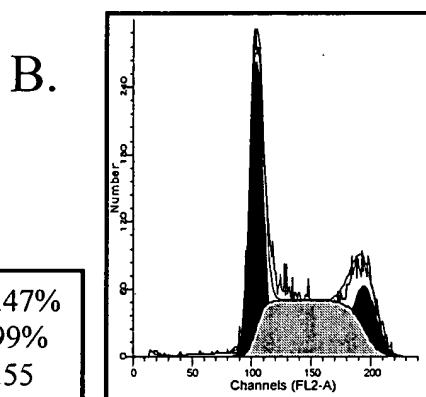
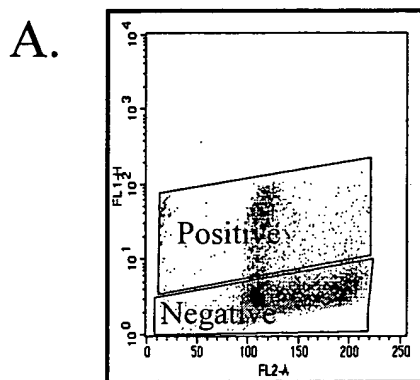


Figure 3 Expression of the stro- μ protein in stromal cells. Western blot analysis using anti-IgM antibody on cell lysates from (1) mouse spleen; (2) overexpression of recombinant stro- μ in 293T cells; (3) MBA-2.1 cells; (4) MEF IgM^{-/-}. As shown in the figure, MBA-2.1 shows a band of 50 kDa protein similar to the recombinant stro- μ expressed in 293T cells.

6. Overexpression of the stro- μ chain in human 293T cells led to G1 phase arrest in the (Figure 4), indicating that stro- μ is functional in these mesenchymal cells upon overexpression. Figure 6 shows FACS results of ectopic stro- μ expression in a tumor cell line, 293T, compared to non-transfected cells. 293T cells, a cellular TK+ cell line, are derived from human kidney epithelial cells transformed with adenovirus E1A and E1B as well as the simian virus 40T antigen. Figure 6B shows that the majority of the non-transfected 293T cells (~46%) are in S phase, with less than 40% of the cells in G1 phase. On the other hand, more than 85% of the stro- μ transfected 293T cells are in G1 phase. These results clearly show that stro- μ is able to induce G1 arrest of a tumor cell line.



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Figure 4 Expression of stro- μ in 293T induces G1 arrest. A. 293T cells, 24h after transfection with stro- μ , stained with anti-IgM. Gated for positive and negative stained cells; B. Cell cycle distribution of 293T non transfected cells 24h after transfection harvested and stained with PI and DNA content; C. 293T non transfected cells 24h after transfection- represented by negatively stained cells by anti-IgM; D. Cell cycle distribution of 293T transfected with stro- μ cells; E. 293T transfected with stro- μ represented by positively stained cells by anti-IgM.

7. The expression of stro- μ induces differentiation of tumor cells, as detected using an anti-CD-10 antibody (Figure 5A). We believe that these results and those presented in figure 4, unequivocally show that expression of stro- μ is useful in treating cancer by inducing G1 arrest and cell differentiation.

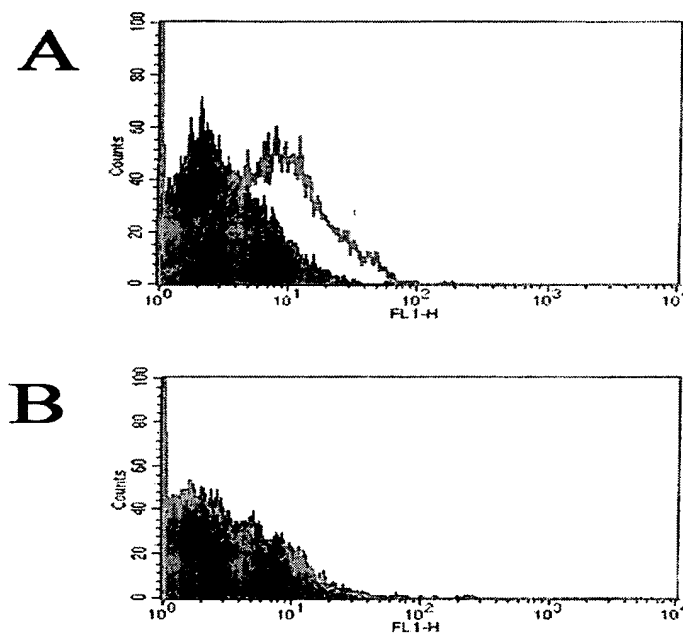


Figure 5 Stro- μ induces upregulation of CD10: FACS analysis of 293T transfected with stro- μ (A) or empty vector (B) and stained with anti-CD10 [green (light color)line] compared to isotype control [purple(dark line)].

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I further declare that all statements made herein are based on my knowledge or information and belief and are believed to be true and that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing there from.

Date 30/8/06

Respectfully submitted,



Dov Zipori